

Anticytomegalovirus antibody titres are not associated with caregiving burden in younger caregivers

Vitlic, Ana; Phillips, Anna C; Gallagher, Stephen; Oliver, Christopher; Lord, Janet M; Moss, Paul

DOI:

[10.1111/bjhp.12092](https://doi.org/10.1111/bjhp.12092)

License:

Other (please specify with Rights Statement)

Document Version

Early version, also known as pre-print

Citation for published version (Harvard):

Vitlic, A, Phillips, AC, Gallagher, S, Oliver, C, Lord, JM & Moss, P 2015, 'Anticytomegalovirus antibody titres are not associated with caregiving burden in younger caregivers', *British Journal of Health Psychology*, vol. 20, no. 1, pp. 68-84. <https://doi.org/10.1111/bjhp.12092>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

This is the pre-peer reviewed version of the following article: Vitlic, A., Phillips, A. C., Gallagher, S., Oliver, C., Lord, J. M. and Moss, P. (2015), Anticytomegalovirus antibody titres are not associated with caregiving burden in younger caregivers. *British Journal of Health Psychology*, 20: 68–84., which has been published in final form at <http://onlinelibrary.wiley.com/doi/10.1111/bjhp.12092>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Checked July 2015

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Running head: Caregiving and CMV

Anti-cytomegalovirus antibody titres are not associated with caregiving burden in younger caregivers.

Ana Vitlic MSc^{1,2}, Anna C. Phillips PhD^{1,2*}, Stephen Gallagher PhD³, Chris Oliver PhD⁴, Janet M. Lord PhD^{2,5}, and Paul Moss MD PhD⁶

¹School of Sport and Exercise Sciences, University of Birmingham, Birmingham, England

²MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Birmingham, Birmingham, England

³School of Psychology, University of Limerick, Limerick, Ireland

⁴School of Psychology, University of Birmingham, Birmingham, England

⁵School of Immunity and Infection, University of Birmingham, Birmingham, England

⁶School of Cancer Sciences, University of Birmingham, Birmingham, England

Word count (exc. figures/tables): 4107

*Requests for reprints should be addressed to Dr Anna C. Phillips, School of Sport and Exercise Sciences, University of Birmingham, B15 2TT, UK. e-mail: A.C.Phillips@bham.ac.uk

The authors had no potential conflicts of interest and the study was funded by the University of Birmingham and Neuroendocrine Immune Networks in Ageing EU Commission Marie Curie Training Network.

Abstract

Objective: This analysis examines whether or not younger caregivers, parents of children with developmental disabilities, differed from controls in terms of cytomegalovirus (CMV) seropositivity and CMV-specific antibody titre.

Secondly, it examined whether any particular socio-demographics, health behaviours, or psychological/caregiving variables were associated with a higher CMV antibody titre among caregivers.

Design: Young caregivers and age- and sex-matched controls were compared with respect to their reported health behaviour and psychosocial status as well as latent virus control.

Methods: 117 parents of children with developmental disabilities and 52 control parents completed standard measures of health behaviours, socio-demographics, perceived stress, depression and anxiety, caregiver burden, child problem behaviours. They also provided a blood sample assayed for presence of CMV-specific antibody.

Results: Caregivers were no more likely to be CMV positive than controls and did not have higher antibody titres against CMV. In addition, there was no association between CMV antibody titre in seropositive caregivers and any of the psychological/caregiving variables. However, higher CMV antibody titres were significantly associated with a higher BMI, lower exercise levels, smoking and lower fruit and vegetable and fat intake among seropositive caregivers.

Conclusions: These data suggest that in the absence of immunosenescence, the chronic stress of caregiving is not sufficient to compromise the immune response to persistent CMV infection. However, an indirect mechanism to

poorer health in caregivers might be via adoption of disadvantageous health behaviours in response to stress.

1. Introduction

Cytomegalovirus (CMV) is a ubiquitous β -herpes virus with prevalence rates of infection as high as 60% (Dowd, Aiello, & Alley, 2009). The means of transmission are through bodily fluids such as saliva, tears, urine and breast milk, and it can also be transmitted sexually. As with any virus from the Herpesviridea family, CMV has the ability to remain in the body in the latent phase, typically for CMV this occurs in myeloid and dendritic cell progenitors, where its activation is initiated by inflammatory factors (Hahn, Jores, & Mocarski, 1998). Clinical symptoms and activation of CMV virus in general is highly unlikely in healthy adults. On the other hand, immunocompromised individuals, such as those with HIV infection and organ transplant patients (Riddell & Greenberg, 1997), can display symptomatic CMV infections (Rasmussen, 1991). CMV infection may also contribute to the age-related decline in immunity, immunosenescence (Olsson et al., 2000; Pawelec & Derhovanessian, 2011), and thus an increased susceptibility to infectious disease and risk of mortality, at least in the very old (Almanzar et al., 2005; Pawelec et al., 2005; Simanek et al., 2011; Strandberg, Pitkala, & Tilvis, 2009). The link between CMV seropositivity and increased mortality was proposed to be due to an increase in proinflammatory cytokines (Forsey et al., 2003; Franceschi, Valensin, Fagnoni, Barbi, & Bonafe, 1999; Licastro F., 2005; Trzonkowski, Mysliwska, Pawelec, & Mysliwski, 2009), though our own data has refuted this by showing that inflammation increased with ageing irrespective of CMV serostatus in one large cohort (Bartlett et al., 2012).

It has been shown that psychosocial factors, such as the chronic stress of caregiving, are associated with immune decrements. For example, older spousal caregivers of a person with dementia exhibit a range of immunological features such

as a decreased percentage of T-lymphocytes (Kiecolt-Glaser et al., 1987), and higher antibody titres to Epstein-Barr virus (EBV) (Kiecolt-Glaser, et al., 1987). In addition, caregivers showed lower levels of salivary antibodies compared to non-caregivers, but this was observed only in the elderly cohort of three age groups (Gallagher et al., 2008), suggesting accelerated immune ageing in individuals experiencing periods of chronic stress

Younger parental caregivers of a child with developmental disability, particularly those who experienced greater child problem behaviours, have also been shown to have significantly lower responses to both influenza and pneumococcal vaccinations compared to sex and age matched controls (Gallagher, Phillips, Drayson, & Carroll, 2009a, 2009b; Pariante et al., 1997). In only one other study were immune decrements shown in younger caregivers compared to controls; caregivers had a lower T helper:suppressor cell ratio than controls (Pariante, et al., 1997), an indicator of early immunosenescence. This suggests that perhaps irrespective of the caregiver's age, caring for someone with severe cognitive and behavioural problems may compromise immunity. In addition, older spousal caregivers of dementia patients showed changes in health behaviours which include changes in diet, smoking behaviour and sleep patterns that can also contribute to and further damage their health (Gallant & Connell, 1998) and may be an indirect mechanism through which caregiving stress impacts on immunity.

What is unknown is whether stress in younger caregivers can specifically compromise the ongoing immune response to CMV. Anti-CMV antibody titre is a good marker of an ongoing immune response such that a higher titre would be indicative of compromised immune function. In one study, older but not younger caregivers had higher serum antibody titres against CMV compared to controls

(Pariante, et al., 1997). However, this in part might be driven by the increased likelihood of CMV infection with ageing or the response to episodes of sub-clinical viral reactivation in older adults (Pawelec & Derhovanessian, 2011). In addition, even though CMV and risky health behaviour changes such as smoking, bad diet and inadequate sleep pattern have all been linked to adverse health consequences, the potential interplay between these factors, to our knowledge, remains unknown. Given the negative effects of caregiving stress on other indices of immunity, this study aimed to examine the influence of caregiving stress on CMV antibody titre in younger caregivers. Further, the present study assessed associations between CMV antibody titre and various indices of health behaviours and other psychosocial variables within the caregivers group. It was hypothesised that caregivers for children with developmental disabilities, particularly those with the greatest caregiver burden or child behaviour problems, would have higher antibody titres against CMV than non-caregivers.

2.1 Method

2.1.1. Participants

One hundred and seventeen parents caring for children with developmental disabilities and 52 parents of normally developing children were recruited to the study and provided a blood sample for CMV analysis. Developmental disability is the term used to describe conditions including but not limited to Autism spectrum disorders and Down syndrome (National Institute of Child Health and Development, National Institute of Health- <http://www.nih.gov/icd/>). These parental caregivers were recruited via invitation letters distributed by their respective disease support group associations and by advertising in associated newsletters, and by direct contact with family support groups. Inclusion criteria

for these parents were: caring for at least one child with Autism Spectrum Disorder (ASD), Angelman, Down, Cornelia de Lange, fragile X, or Smith-Magenis syndromes. These disorders evidence a range of problem behaviours, which are particularly common among children with syndromes other than Down (Arron, Oliver, Moss, Berg, & Burbidge, 2011; Blacher & McIntyre, 2006; Chadwick, Piroth, Walker, Bernard, & Taylor, 2000). Since the emotional reaction of parental caregivers is highly influenced by the diagnostic process (Graungaard & Skov, 2007), we aimed to avoid this particular event and focus on the parents' stressful experiences of caring *per se*. Thus, in keeping with existing research (Hastings, Daley, Burns, & Beck, 2006), children with developmental disabilities had to be aged between 3 and 19 years and living at home during the school term. The majority of parents reported caring for a child with Angelman syndrome (28.3%) and Smith-Magenis syndrome (25.7%); followed by the parents of a child with fragile X syndrome (17.7%) and Autistic Spectrum Disorder (ASD) (15%); the remainder were caring for a child with Cornelia de Lange (8.8%), or Down (4.4%) syndromes. Controls, i.e. parents of typically developing children who were in the same age range as the sample of children with disabilities, were recruited via posters in local schools, the university, and the local area (e.g. sports centres and clubs). None reported suffering from an ongoing chronic immune disease, being acutely ill, taking immunosuppressive medication, or reported being pregnant in case of female participants. Attempts were made to match the groups as closely as possible on age, sex, socioeconomic position, ethnicity, and marital status, by recruiting individual parents of normally developing children that matched as near as possible individual parents of children with developmental disabilities. The total

time period for the recruitment of the controls was less than three years. All participants provided written informed consent and the studies had ethical approval from the appropriate local research ethics committees.

2.2 Study design and procedure

The current sample size was comprised of two separate groups of participants that were part of two separate younger caregiving studies focussing on different elements of immune function. The first was a part of a prospective case-control vaccination response study involving three testing sessions; details elsewhere (Gallagher, et al., 2009b). The analysis of CMV status and antibody titre and caregiving variables reported from that study involved baseline sampling only where parents completed questionnaires and then provided a blood sample. The second study was part of a cross-sectional assessment of neutrophil function comprising one blood sampling session.

2.3 Questionnaires

2.3.1 Depression and anxiety

The Hospital Anxiety and Depression Scale (HADS) was used for measuring psychological morbidity (Zigmond & Snaith, 1983). The scale consists of two subscales with seven items in each, one assessing anxiety and the other largely anhedonic aspects of depression. The answers are scored from 0 (not present) to 3 (considerable). Scores for both scales had a range from 0 - 21, with the scores 0-7 being classed as normal, 8-14 as moderate and 15-21 as severe depression and anxiety respectively. The HADS has good concurrent validity (Bramley, Easton, Morley, & Snaith, 1988; Herrmann, 1997), , and boasts good internal consistency,

Cronbach's α of .90 for depression and .93 for anxiety (Moorey et al., 1991); and test-retest reliability, .85 for depression and .84 for anxiety (Herrmann, 1997). The internal consistency in this sample was .78 for depression and .80 for anxiety.

2.3.2 Time spent caregiving

Amount of time spent caregiving was assessed using a modified version of the Caregiver Activity Survey (Davis et al., 1997). Parents were asked how much time they spend on five specific (transport, dressing, eating, bathing and supervision) caring activities. The total daily score for time spent caregiving was obtained by summarizing hours for each caring role.

2.3.3. Caregiver Burden

Parental caregiver burden was assessed using an adapted form of the Zarit Burden Interview (Zarit, Reever, & Bachpeterson, 1980), originally designed to assess the burden experienced by family caregivers of elderly persons with dementia. Questions in the scale used in the current study had 'your relative' amended with 'your child', for example 'Overall, how burdened do you feel in caring for your child? Responses ranged from 0, never, to 4, nearly always, and the overall score ranges from 0 to 48. Internal consistency in current sample was .93.

2.3.4. Child behaviour difficulties and problems

Child behaviour difficulties were measured using the Strengths and Difficulties Questionnaire (Goodman, 1997). Questions are rated as 0, not true, 1, somewhat true, or 2, certainly true, and higher score indicates more problem

behaviour. Some items are reversed scored (e.g. generally liked by other children, has at least one good friend). The overall score for child problematic behaviours can vary from 0 to 50. The scale has been shown to be reliable (Cronbach's $\alpha = .76$) and effective at identifying behavioural problems in children (Goodman & Scott, 1999) and has been used extensively in research with children with developmental disabilities (Hastings, et al., 2006). Internal consistency for the scale in this study was .80.

2.3.5 Perceived stress

This 14-item stress scale assessed control over and overload with the daily life stress during the past month. It has been frequently used in caregiver research (Glaser, Sheridan, Malarkey, MacCallum, & Kiecolt-Glaser, 2000; Vedhara et al., 2002), and measures both participants' subjective feeling of how much control they feel they have over daily events, as well as their inability to cope with things. The scale ranges from 0 to 4 and higher scores indicate higher perceived stress. The overall score can range from 0 to 56. The scale shows good test–retest reliability ($r = .80$) and internal reliability (Cronbach's $\alpha = .75$). Internal reliability in the present sample was 0.79.

2.3.6 Social support

The 12-item Support Functions Scale – short form (Dunst, 1988) was used to assess types of support available to parents. The support is assessed by 5-point Likert scale with 1 meaning support is not available and 5 that it is available quite often. The total score on this scale varies from 0 to 60. This scale has been shown to be reliable (Cronbach's $\alpha = .86$) and has been used

previously in developmental disability research (White N, 2004). In the present study internal consistency was 0.88.

2.3.7. Sleep

Sleep quality was measured by the 19-item Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989). These 19-items are then combined to create seven component scores with scores ranging from 0, no problems in area, to 3, high problem area. Higher total scores indicated poorer sleep quality. Adequate internal consistency (Cronbach's $\alpha = .69$) has been demonstrated previously (Spira et al., 2012). In the present sample, the Cronbach's $\alpha = .77$.

2.3.8. Health behaviours

A questionnaire adapted from the Whitehall II study (Marmot et al., 1991) was used to assess the health behavior in parents. This questionnaire has been consistently used in previous stress and immunity research (Burns, Carroll, Drayson, Whitham, & Ring, 2003; Phillips, Burns, Carroll, Ring, & Drayson, 2005). Parents were asked about their sleep, smoking status, how much alcohol they drank, and simple categorical scoring system was used in all cases. They were also asked about their exercise engagement (if and how many hours are they involved in the mildly, moderate, or vigorous exercise). They also reported consumption of various food items, which gave a measure of how healthy (fruit and vegetable), or high in fat (snacks, chips, processed meat) their diet was. Categorically scored health behaviour variables with little overall variability were split at the median and converted to binary variables e.g. smoker

versus non-smoker, > 20 alcohol units consumed per week. Fruit and vegetable and fat intake were scored from 0 'never' to 6 'more than once per day' for a range of foods consumed per week; fruit and vegetables listed were then summed along with fatty foods to produce two separate scores. Exercise frequency was scored categorically from 0 'none' to 5 '11+ hours per week' for mild, moderate, and vigorous activity. A total weighted score was then produced from mild + (moderate * 2) + (vigorous * 3).

All of the caregiver measures were also appropriate to parents of non-developmentally disabled children, as they cover the general parental caring role and child behaviours.

2.4 Blood sampling and CMV analysis

Venous blood was collected from an ante-cubital vein into a plain 7ml tube (BD Vacutainer, Meylan Cedex). The samples were allowed to clot for at least one hour, were centrifuged at 3000 rpm (MSE Centaur) for 10 min, and the separated serum was frozen at -20 °C until assayed. Serum was analysed for the presence of CMV infection using CMV a standardised ELISA developed by the Antiviral Immunology lab, Cancer Sciences, University of Birmingham as previously reported (Savva G M, 2013; Wall et al., 2013). The standard curve measured up to 1000 arbitrary units of IgG and those with more than 10 units were considered CMV positive.

2.5 Statistical analyses

Based on our previous caregiver research we attempted to detect a medium sized effect ($f = 0.29$) giving an overall sample size of $N = 96$, with at least 48

participants in each group. Chi-square and ANOVA were used to determine group differences (caregiver versus control) in health behaviour, socio-demographics and psychosocial variables. Any significant group differences in socio-demographic variables and health behaviours were controlled for in subsequent analyses. Prior to statistical analysis of CMV data, these were checked for normality. Due to the skew of the data, antibody titres were subjected to \log_{10} transformation. Four caregivers and two controls were removed from analyses due to outlying CMV antibody values or finding out post-consent that their child was < 3 or > 19 years. Chi-square was used to establish whether CMV status differed between the groups (caregiver versus control) or related to any socio-demographic, health behaviour, or psychological/caregiving variables. ANOVA was used to compare CMV antibody titre between the caregivers and controls among those who were seropositive only. For CMV titre, among the seropositive participants only, correlations were conducted within the caregiver group alone to determine if any of descriptive, health behaviour or psychological/caregiving variables related to antibody titre among those with the chronic stress of caregiving. For all analyses, effect size (ϕ , η^2 , r) was reported for all the analyses conducted, followed by observed power. Any variations in degrees of freedom reflect occasional missing data.

3. Results

3.1 Socio-demographic, childcare and psychosocial characteristics of parental groups

The demographic and summary childcare characteristics of the two parental groups are presented in Table 1. Caregivers and controls did not differ on key

socio-demographics , but parents of children with developmental disabilities had a higher BMI, depression and anxiety symptoms, perceived stress, alcohol intake, fat intake, poorer sleep quality, and spent more time caregiving. As might be expected, parents caring for a child with a developmental disability had a greater caregiving burden, and their children had more child behaviour difficulties than parents of typically developing children (see Table 1). Further, social support questionnaires revealed poorer quality of support (Support Function Scale) than that received by controls.

[Insert Table 1 about here]

3.2 CMV status

Of the group as a whole, 76 (47%) participants were CMV positive; the number CMV seropositive within each group is shown in Table 1. The percentage of CMV positive individuals among caregivers and controls were almost identical, $\chi^2(1) = .01$, $p = .92$, $\phi = .008$, $<.26$. Overall, CMV status was also not associated with any of the socio-demographic, health behaviour, or any of the psychological/caregiving variables.

3.3 Caregiving and CMV antibody titre

For the CMV positive subset (N = 76), socio-demographic, caregiving and psychosocial characteristics are presented in Table 2. This time, in addition to higher alcohol intake, higher BMI, depression and anxiety symptoms, higher perceived stress and lower social support, caregiving parents differed in occupational group (more likely to be manual), ethnicity (more likely to be

White), and smoking behaviour (more likely to be smokers) compared to control parents, but showed similar fruit and vegetable and fat intake. Although CMV positive caregivers had higher CMV antibody titres than controls, the difference was not statistically significant, even after controlling for the significant socio-demographic and health behaviour variables, $F(1,50) = 0.14$, $p = .71$, $\eta^2 = .003$, $1-\beta = .06$, and significant psychosocial variables, $F(1,60) = 1.73$, $p = .19$, $\eta^2 = .028$, $1-\beta = .25$.

3.4 Characteristics of all participants and CMV antibody titre

CMV antibody titre overall was also not associated with most of the socio-demographic, health behaviour, and psychological/caregiving variables, although women had significantly higher titres $F(1,69) = 4.38$, $p = .04$, $\eta^2 = .060$, $1-\beta = .54$, as did those with higher BMI, $r(64) = .26$, $p = .03$, $.69$. Adjustment for sex and BMI as covariates did not alter the previous lack of group difference.

[Insert Table 2 about here]

3.5 Characteristics within the caregiving group and CMV antibody titre

When seropositive caregivers were considered as a group, there was no association between CMV antibody titre and age, age of child, sex, ethnicity, occupational status, marital status, or sleep quality. However, there was a positive correlation between CMV antibody titre and BMI, $r(44) = .29$; $p = .05$, $.63$, as observed in the whole group, and smokers had higher antibody titre than non-smokers, $F(1,47) = 5.92$, $p = .019$, $\eta^2 = .112$, $1-\beta = .66$. Other variables that showed significant associations with CMV antibody titre in the caregivers group were fruit and vegetable consumption, $r(47) = -.28$, $p = .048$, $.63$, fat intake,

$r(46) = -.34, p = .019, .63$, and exercise score, $r(42) = -.37, p = .013, .61$, such that those who consumed fewer fruit and vegetables and fat, and undertook less frequent exercise had higher CMV antibody titres. There were no associations between CMV antibody titre and psychological/caregiving variables, as shown in Table 3.

[Insert Table 3 about here]

4. Discussion

Despite differing on all main psychological/caregiving variables but not on socio-demographics, parents caring for children with developmental disabilities were no more likely to be CMV seropositive than parents of typically developing children. Caregivers were also no more likely to have significantly higher antibody titres against CMV. Finally, although CMV antibody was not associated with any of the psychological/caregiving variables or most socio-demographics, women and people with higher BMI had higher antibody titres. This gender difference is consistent with previous research (Haarala et al., 2012; McVoy & Adler, 1989), the exact reason being unknown. One possibility might be that hormonal changes, specifically the increase in oestrogen that occurs during pregnancy relates to reactivation of CMV, and consequently higher titres later in life (Kleinman, Sarov, & Insler, 1986; McVoy & Adler, 1989). Higher CMV antibody titres in those with higher BMI is also in concordance with a previously reported finding in immunocompetent adults (Gkrania-Klotsas et al., 2013). This is perhaps due to associations noted between obesity and inflammation (Forsythe, Wallace, & Livingstone, 2008) and CMV and inflammation

(Hummel & Abecassis, 2002), although this has not been observed in all studies (Bartlett, et al., 2012).

Within the seropositive caregiving parents, there was no association between CMV antibody titre and any of the psychological/caregiving variables. However, those with higher CMV antibody titres, potentially indicating poorer latent virus control, generally did have poorer health behaviours, including being more likely to smoke, have a higher BMI, intake less fruit and vegetables, and undertake less exercise.

This finding is consistent with that of Pariante et al. (1997), who did not find a greater likelihood of CMV infection or higher titres among the younger age range of their caregiving sample, as well as with studies in caregivers for a person with a physical disorder which found no group differences in comparison to controls (Baron, Cutrona, Hicklin, Russell, & Lubaroff, 1990; Epel et al., 2004; Provinciali et al., 2004). These studies suggest that younger caregivers and those caregiving for a more physical than mental disorder are less vulnerable to immune decrements. However, the present findings contrast with the other caregiving and immunity studies in younger adults, which suggested poorer immunity among young caregivers, at least in response to vaccination (Gallagher, et al., 2009a, 2009b; Gennaro et al., 1997) and lymphocyte proliferation to mitogen. This may reflect greater resilience of the CD8 T cell mediated anti-viral immune response to stress, compared with the many components of the vaccination response which include macrophages and dendritic cells in the skin as well as T and B cell co-operation to generate antibody.

A similar percentage of CMV positive parents in both caregiving (47%) and control (46%) groups indicated no difference in infection rate consistent with the fact that disparities in CMV infection rate among parents are mainly attributed to ethnicity and income (Colugnati, Staras, Dollard, & Cannon, 2007), which did not differ between the groups. In addition, other studies have shown that children in day care have a higher CMV prevalence (Pass & Hutto, 1986), which might in turn lead to greater infection in their parents. However, information regarding day care or children's CMV titres was not available in the present study. Nevertheless, exposure to CMV-shedding children alone is not sufficient for infection in parents (Yamashita, Fujimoto, Nakajima, Isagai, & Matsuishi, 2003), thus parents exposed to children with developmental disabilities who may have a high burden of infection themselves (Arron, et al., 2011; do Canto CL, 2000) are not necessarily more likely to be seropositive.

Caregiving and control groups also had comparable CMV antibody titres. The determinants of CMV-specific antibody titre are unclear but are likely to be related to the frequency and magnitude of subclinical viral replication *in vivo*. In addition to that, a high level of circulating antibody will not prevent reactivation of the virus (Glaser R, 1994), but rather indicate that it has likely been reactivated. Viral reactivation events are believed to become more common with increasing age and it is of interest that an effect of caregiving stress and burden on CMV antibody titre was noted in a study of older adults (Pariante, et al., 1997) in contrast to the present younger caregiving group. This perhaps indicates that in earlier life, caregiving *per se* is not sufficient to induce significant immune decrement seen here as greater viral reactivation, which is again consistent with some studies in

young caregivers (Epel, et al., 2004; Gallagher, et al., 2008; Vedhara, et al., 2002), but in contrast with others (Gallagher, et al., 2009a, 2009b). Testing of the age x caregiver group interaction in this study did not reveal any significant interaction effect. Further, although Pariante et al. demonstrated a higher antibody titre in caregivers when compared to the controls in their eldest cohort, the sample size used for the analysis, was only 18 female participants which was further reduced to 9 participants in each group after splitting by age. Therefore, it could be argued that the evidence for poorer control over this latent virus in older caregivers is only weak.

CMV is also suggested to have a role in the ageing of the immune system, and has been used to explain the difference in the various immune components between CMV-infected and CMV non-infected elderly (Pawelec, Derhovanessian, Larbi, Strindhall, & Wikby, 2009). In addition, older caregivers of Alzheimer's patients showed dramatic increase in antibody titre to EBV, another latent virus from Herpesviridae family, pointing to impaired control of cellular immunity towards virus replication (Kiecolt-Glaser, Speicher, Glaser, Dura, & Trask, 1991). This suggests that as caregivers age, they are at higher risk of poorer latent virus control and its consequences.

Among CMV seropositive caregivers, smokers had higher antibody titre than non-smokers. This is not surprising as the T and B cell response against different antigens is severely reduced by smoking (Holt & Keast, 1977; Sopori, 2002), which could influence susceptibility to infection (Evans et al., 2000), and perhaps in this particular case, control of latent virus reactivation. Other health behaviour characteristics that were associated with CMV titre were fruit and vegetable and fat

intake and amount of exercise. To our knowledge there is no previous evidence of the effects of dietary intake on latent virus control. Thus, what we may be picking up on here are a range of negative health behaviours which have an impact on a several health outcomes and processes, including a flattening in the diurnal rhythm of cortisol similar to that observed in immunosenescence (Heaney, Phillips, & Carroll, 2012) and now control of CMV reactivation. Further, an indirect mechanism between the stress of caregiving and the poor health reported in caregivers under high levels of stress and burden (Forbes, While, & Mathes, 2007) might be via engagement in negative health behaviours such as poor diet and lack of exercise, resulting in poorer immunity as well as worse health generally. However, longitudinal studies including markers of health and more thorough assessment of health behaviours in caregivers would need to be conducted in order to test this theory in depth. That these associations with CMV titre only emerged in the caregiver group rather than overall might be due to the increased importance of these behaviours in combination with existing stress. This argument has been made previously with regard to the emerging impact of a psychosocial/behavioural factor in combination with an existing source of stress which itself had no direct impact on immunity. For example, only older hip fracture patients who developed depression showed suppressed immunity, rather than those who had the stress of hip fracture alone (Duggal, Upton, Phillips, & Lord, 2013). In younger non-stressed groups, although still detrimental in the long term, these health behaviours might not demonstrate their impact on particular aspects of immunity until later in life.

The present study has a number of limitations. First, even though the initial sample size might be regarded as small, it was substantial when compared with the other caregiving studies (Gallagher, et al., 2009b; Pariante, et al.,

1997; Vedhara, et al., 2002). We also recruited fewer controls than caregivers, as our key interest lay in the potential associations between elements of caregiver stress and CMV titre which might have explained any caregiver-control group differences. However, once focusing on seropositive caregivers our sample size has significantly decreased and as such may have limited power, but, again, it is of similar magnitude or larger than other caregiver studies (Glaser, et al., 2000; Pariante, et al., 1997; Vedhara et al., 1999) and we have reported observed power throughout. Nonetheless, the number of tests might have increased the likelihood of a Type I error. Second, even though antibody titre has been commonly used as a measure of the adequacy of an individual's immune system to control latent virus, it is known that cellular immunity and the T cell response in particular are key components needed for suppression of CMV reactivation (Pawelec & Derhovanessian, 2011; Vasto et al., 2007). However, such assays require large quantities of whole blood and for the present analyses only stored sera were available. Third, assessment of other antibodies besides IgG such as IgM would be useful in order to ascertain recency of infection and reinfection in caregivers and controls as well as reactivation, but we did not have data on IgM levels. Fourth, this study was cross-sectional at one time point, and therefore unable to determine whether comparable CMV antibody titres between the groups were indeed a consequence of adequate latent virus control in the stressed group, or they were related to other factors such as different times of initial infection. Measuring burden and CMV antibody titre over several time points in future research would help to clarify this. For example, in a study of antibody

titres against EBV between older caregivers and controls, comparable titres were found at baseline but a greater increase was observed in the caregiver group over time (Kiecolt-Glaser, et al., 1991). Finally, there is an inevitable limitation in the method of recruitment for this study, as the contact with caregiving parents was made mainly via family support groups and on the particular syndrome conference days, thus might have been biased towards those caregivers who have an access to adequate support and therefore, at least in part, obtain relief from the burden of caregiving. However, in our experience, those who attend syndrome days in order to gain relief would not have this support available to them generally in their daily life, thus are still a very stressed group.

In conclusion, there was no evidence of an association between caregiving and CMV seropositivity or anti-CMV antibody titres. This suggests that caregiving in younger adults may not accelerate all elements of immunosenescence, but that such decrements are only observed in older caregivers. However, there was some evidence of an association between caregivers engaging in unhealthy behaviours (dietary intake, exercise and BMI) and CMV antibody titre, suggesting that an unhealthy lifestyle in response to stress in some individuals could be an indirect pathway through which health is affected in caregivers.

Table 1. Socio-demographics, childcare and psychosocial characteristics of each group

	Mean (SD) / N (%)		Analyses
	Caregivers (N = 113)	Controls (N = 50)	
Socio-demographic characteristics			
Sex (Female)	70 (67%)	30 (61%)	$X^2(1) = .54, p = .46, \phi = .060, .23$
Marital status (Partnered)	90 (87%)	40 (82%)	$X^2(1) = .63, p = .43, \phi = .064, .23$
Ethnicity (Caucasian)	96 (92%)	42 (86%)	$X^2(1) = 1.64, p = .20, \phi = .103, .23$
Occupational status (non-manual)	66 (71%)	38 (79%)	$X^2(1) = 1.10, p = .29, \phi = .088, .22$
Age (years)	40.8 (6.83)	40.2 (4.72)	$F(1,151) = .301, p = .58, \eta^2 = .002, 1-\beta = .17$
Health behaviour characteristics			
Smoking (smoker)	37 (36%)	19 (40%)	$X^2(1) = 0.28, p = .60, \phi = .043, .23$
Alcohol (>20 units per week)	23 (22%)	1 (2%)	$X^2(1) = 9.67, p = .002, \phi = .253, .69$
Body Mass Index (kg/m ²)	26.7 (5.40)	24.3 (3.14)	$F(1,142) = 7.45, p = .007, \eta^2 = .050, 1-\beta = .77$
Fruit and vegetable intake score	13.7 (3.92)	12.8 (3.38)	$F(1,149) = 2.21, p = .14, \eta^2 = .015, 1-\beta = .32$
Fat intake score	19.9 (6.97)	17.0 (8.06)	$F(1,149) = 5.02, p = .03, \eta^2 = .033, 1-\beta = .61$

Exercise score	10.1 (6.31)	10.4 (6.22)	$F(1,140) = 0.95, p = .76, \eta^2 = .001, 1-\beta = .06$
Sleep total score	9.3 (3.21)	7.1 (2.83)	$F(1,140) = 17.09, p < .001, \eta^2 = .109, 1-\beta = .98$
Number CMV seropositive	53 (47%)	23 (46%)	$\chi^2(1) = 0.01, p = .92, \phi = .008, <.25$
Raw CMV antibody titre	110.2 (162.97)	92.3 (142.98)	$F(1,161) = 0.45, p = .50, \eta^2 = .003, 1-\beta = .10$
Psychosocial/caregiving characteristics			
Age of main care recipient (years)	9.1 (4.14)	7.9 (4.34)	$F(1,161) = 2.95, p = .09, \eta^2 = .018, 1-\beta = .40$
Hours spent caregiving per day	12.5 (9.48)	6.8 (12.50)	$F(1,148) = 9.63, p = .002, \eta^2 = .061, 1-\beta = .87$
HADS depression	8.5 (3.66)	4.0 (3.29)	$F(1,149) = 51.54, p < .001, \eta^2 = .257, 1-\beta = 1.00$
HADS anxiety	10.3 (4.26)	5.9 (2.52)	$F(1,151) = 45.13, p < .001, \eta^2 = .230, 1-\beta = 1.00$
Perceived stress	30.3 (7.33)	23.6 (6.47)	$F(1,151) = 30.00, p < .001, \eta^2 = .166, 1-\beta = 1.00$
Caregiver burden score (BI)	27.3 (9.10)	13.0 (7.58)	$F(1,151) = 53.43, p < .001, \eta^2 = .261, 1-\beta = 1.00$
Child behaviour difficulties (SDQ)	19.3 (5.17)	7.7 (4.34)	$F(1,150) = 182.41, p < .001, \eta^2 = .549, 1-\beta = 1.00$
Social support	30.1 (9.01)	37.9 (10.48)	$F(1,147) = 22.25, p < .001, \eta^2 = .131, 1-\beta = 1.00$

Table 2. Socio-demographics, childcare and psychosocial characteristics between CMV seropositive subsets of parents of children with developmental disabilities and control parents.

	Mean (SD) / N (%)		<i>Analyses</i>
	Caregivers (N = 53)	Controls (N = 23)	
Sex (Female)	35 (71%)	13 (59%)	$X^2(1) = 1.06, p = .30, \phi = .122, <.20$
Marital status (Partnered)	41 (84%)	16 (73%)	$X^2(1) = 1.15, p = .28, \phi = .127, <.20$
Ethnicity (Caucasian)	46 (94%)	17 (77%)	$X^2(1) = 4.19, p = .041, \phi = .243, .39$
Occupational status (non-manual)	24 (57%)	18 (86%)	$X^2(1) = 5.15, p = .023, \phi = .286, .64$
Age (years)	42.1 (5.99)	40.6 (5.05)	$F(1,69) = 1.00, p = .32, \eta^2 = .014, 1-\beta = .166$
Smoking (smoker)	25 (51%)	5 (24%)	$X^2(1) = 4.44, p = .035, \phi = .252, .39$
Alcohol (>20 units per week)	9 (18%)	0 (0%)	$X^2(1) = 4.43, p = .035, \phi = .251, .39$
Body Mass Index (kg/m ²)	26.8 (3.85)	23.8 (2.87)	$F(1,64) = 9.51, p = .003, \eta^2 = .129, 1-\beta = .859$
Fruit and vegetable intake score	12.9 (4.18)	13.6 (2.73)	$F(1,69) = 0.54, p = .46, \eta^2 = .008, 1-\beta = .112$
Fat intake score	17.2 (7.67)	19.5 (7.21)	$F(1,68) = 1.40, p = .24, \eta^2 = .020, 1-\beta = .214$

Exercise score	9.0 (6.71)	10.6 (4.92)	$F(1,64) = 1.00, p = .32, \eta^2 = .015, 1-\beta = .166$
Sleep total score	9.6 (3.48)	6.8 (2.62)	$F(1,65) = 10.95, p = .002, \eta^2 = .144, 1-\beta = .903$
Raw CMV antibody titre	234.1 (166.50)	199.5 (152.49)	$F(1,74) = 0.73, p = .40, \eta^2 = .010, 1-\beta = .134$
Age of main care recipient (years)	9.7 (4.37)	7.8 (4.53)	$F(1,74) = 3.06, p = .08, \eta^2 = .040, 1-\beta = .408$
Hours spent caregiving per day	12.1 (8.04)	10.1 (17.62)	$F(1,67) = 0.44, p = .51, \eta^2 = .006, 1-\beta = .100$
HADS depression	8.7 (3.76)	3.5 (2.97)	$F(1,69) = 32.44, p < .001, \eta^2 = .320, 1-\beta = 1.00$
HADS anxiety	10.2 (4.08)	5.5 (2.06)	$F(1,69) = 26.04, p < .001, \eta^2 = .274, 1-\beta = .999$
Perceived stress	30.3 (7.58)	22.7 (5.20)	$F(1,69) = 18.33, p < .001, \eta^2 = .210, 1-\beta = .988$
Caregiver burden score (BI)	34.1 (14.44)	14.9 (7.94)	$F(1,69) = 34.19, p < .001, \eta^2 = .331, 1-\beta = 1.00$
Child behaviour difficulties (SDQ)	20.4 (5.18)	6.8 (4.45)	$F(1,68) = 110.38, p < .001, \eta^2 = .619, 1-\beta = 1.00$
Social support	28.9 (8..93)	36.0 (10.90)	$F(1,67) = 8.22, p = .006, \eta^2 = .109, 1-\beta = .807$

Table 3. Spearman's rho correlations among CMV titers and the caregivers' psychosocial characteristics

	r	df	p
HADS depression	.03	47	.81
HADS anxiety	.03	47	.82
Perceived stress	-.11	47	.44
Hours spent caregiving per day	-.14	45	.35
Caregiver burden score (BI)	.13	47	.37
Child behaviour difficulties (SDQ)	-.04	47	.77
Social support	.12	46	.42

5. References

- Almanzar, G., Schwaiger, S., Jenewein, B., Keller, M., Herndler-Brandstetter, D., Wurzner, R., et al. 2005. Long-term cytomegalovirus infection leads to significant changes in the composition of the CD8(+) T-cell repertoire, which may be the basis for an imbalance in the cytokine production profile in elderly persons. *Journal of Virology*, 79, 3675-3683. doi: 10.1128/jvi.79.6.3675-3683.2005.
- Arron, K., Oliver, C., Moss, J., Berg, K., & Burbidge, C. 2011. The prevalence and phenomenology of self-injurious and aggressive behaviour in genetic syndromes. *Journal of Intellectual Disability Research*, 55, 109-120. doi: 10.1111/j.1365-2788.2010.01337.x.
- Baron, R. S., Cutrona, C. E., Hicklin, D., Russell, D. W., & Lubaroff, D. M. 1990. Social support and immune function among spouses of cancer patients. *Journal of Personality and Social Psychology*, 59, 344-352. doi: 10.1037/0022-3514.59.2.344.
- Bartlett, D. B., Firth, C. M., Phillips, A. C., Moss, P., Baylis, D., Syddall, H., et al. 2012. The age-related increase in low-grade systemic inflammation (Inflammaging) is not driven by cytomegalovirus infection. *Aging Cell*, 11, 912-915. doi: 10.1111/j.1474-9726.2012.00849.x.
- Blacher, J., & McIntyre, L. L. 2006. Syndrome specificity and behavioural disorders in young adults with intellectual disability: cultural differences in family impact. *Journal of Intellectual Disability Research*, 50, 184-198. doi: 10.1111/j.1365-2788.2005.00768.x.
- Bramley, P. N., Easton, A. M. E., Morley, S., & Snaith, R. P. 1988. The differentiation of anxiety and depression by rating scales. *Acta Psychiatrica Scandinavica*, 77, 133-138. doi: 10.1111/j.1600-0447.1988.tb05089.x.
- Burns, V. E., Carroll, D., Drayson, M., Whitham, M., & Ring, C. 2003. Life events, perceived stress and antibody response to influenza vaccination in young healthy adults. *Journal of Psychosomatic Research*, 55, 569-572. doi:
- Buyse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. 1989. The Pittsburgh sleep quality index - a new instrument for psychiatric practice and research. *Psychiatry Research*, 28, 193-213. doi: 10.1016/0165-1781(89)90047-4.
- Chadwick, O., Piroth, N., Walker, J., Bernard, S., & Taylor, E. 2000. Factors affecting the risk of behaviour problems in children with severe intellectual disability. *Journal of Intellectual Disability Research*, 44, 108-123. doi: 10.1046/j.1365-2788.2000.00255.x.
- Colugnati, F. A. B., Staras, S. A. S., Dollard, S. C., & Cannon, M. J. 2007. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *Bmc Infectious Diseases*, 7 doi: 10.1186/1471-2334-7-71.
- Davis, K. L., Marin, D. B., Kane, R., Patrick, D., Peskind, E. R., Raskind, M. A., et al. 1997. The Caregiver Activity Survey (CAS): Development and validation of a new measure for caregivers of persons with Alzheimer's disease. *International Journal of Geriatric Psychiatry*, 12, 978-988. doi: 10.1002/(sici)1099-1166(199710)12:10<978::aid-gps659>3.0.co;2-1.
- do Canto CL, G. C., Garcez E, Villas Boas LS, Fink MC, Estevam MP, Pannuti CS. 2000. Cytomegalovirus infection in children with Down syndrome in a day-care center in Brazil. *Rev Inst Med Trop Sao Paulo*., 42, 179-183. doi:
- Dowd, J. B., Aiello, A. E., & Alley, D. E. 2009. Socioeconomic disparities in the seroprevalence of cytomegalovirus infection in the US population: NHANES III. *Epidemiology and Infection*, 137, 58-65. doi: 10.1017/s0950268808000551.

- Duggal, N. A., Upton, J., Phillips, A. C., & Lord, J. M. 2013. Depression is associated with reduced neutrophil function in hip fracture patients. . *Brain, Behavior & Immunity*, 33, 173-182. doi: 10.1016/j.bbi.2013.07.004.
- Dunst, C., Trivette, CM, Deal, AG. 1988. Enabling and empowering families: principles and guidelines for practice. . Cambridge: MA: Brookline Books.
- Epel, E. S., Blackburn, E. H., Lin, J., Dhabhar, F. S., Adler, N. E., Morrow, J. D., et al. 2004. Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 17312-17315. doi: 10.1073/pnas.0407162101.
- Evans, P., Der, G., Ford, G., Hucklebridge, F., Hunt, K., & Lambert, S. 2000. Social class, sex, and age differences in mucosal immunity in a large community sample. *Brain Behavior and Immunity*, 14, 41-48. doi: 10.1006/brbi.1999.0571.
- Forbes, A., While, A., & Mathes, L. 2007. Informal carer activities, carer burden and health status in multiple sclerosis. *Clinical Rehabilitation*, 21, 563-575. doi: 10.1177/0269215507075035.
- Forsey, R. J., Thompson, J. M., Ernerudh, J., Hurst, T. L., Strindhall, J., Johansson, B., et al. 2003. Plasma cytokine profiles in elderly humans. *Mechanisms of Ageing and Development*, 124, 487-493. doi: 10.1016/s0047-6374(03)00025-3.
- Forsythe, L. K., Wallace, J. M., & Livingstone, M. B. 2008. Obesity and inflammation: the effects of weight loss. *Nutr Res Rev*, 21, 117-133. doi: S0954422408138732 [pii] 10.1017/S0954422408138732.
- Franceschi, C., Valensin, S., Fagnoni, F., Barbi, C., & Bonafe, M. 1999. Biomarkers of immunosenescence within an evolutionary perspective: the challenge of heterogeneity and the role of antigenic load. *Experimental Gerontology*, 34, 911-921. doi: 10.1016/s0531-5565(99)00068-6.
- Gallagher, S., Phillips, A. C., Drayson, M. T., & Carroll, D. 2009a. Caregiving for Children With Developmental Disabilities Is Associated With a Poor Antibody Response to Influenza Vaccination. *Psychosomatic Medicine*, 71, 341-344. doi: 10.1097/PSY.0b013e31819d1910.
- Gallagher, S., Phillips, A. C., Drayson, M. T., & Carroll, D. 2009b. Parental caregivers of children with developmental disabilities mount a poor antibody response to pneumococcal vaccination. *Brain Behavior and Immunity*, 23, 338-346. doi: 10.1016/j.bbi.2008.05.006.
- Gallagher, S., Phillips, A. C., Evans, P., Der, G., Hunt, K., & Carroll, D. 2008. Caregiving is associated with low secretion rates of immunoglobulin A in saliva. *Brain Behavior and Immunity*, 22, 565-572. doi: 10.1016/j.bbi.2007.11.007.
- Gallant, M. P., & Connell, C. M. 1998. The stress process among dementia spouse caregivers - Are caregivers at risk for negative health behavior change? *Research on Aging*, 20, 267-297. doi: 10.1177/0164027598203001.
- Gennaro, S., Fehder, W. P., Cnaan, A., York, R., Campbell, D. E., Gallagher, P. R., et al. 1997. Immune responses in mothers of term and preterm very-low-birth-weight infants. *Clinical and Diagnostic Laboratory Immunology*, 4, 565-571. doi: 10.1093/cid/cit083.
- Gkrania-Klotsas, E., Langenberg, C., Sharp, S. J., Luben, R., Khaw, K. T., & Wareham, N. J. 2013. Seropositivity and Higher Immunoglobulin G Antibody Levels Against Cytomegalovirus Are Associated With Mortality in the Population-Based European Prospective Investigation of Cancer-Norfolk Cohort. *Clinical Infectious Diseases*, 56, 1421-1427. doi: 10.1093/cid/cit083.
- Glaser R, K.-G. J. 1994. Stress-associated immune modulation and its implications for reactivation of latent herpesviruses. In J. J. Glaser R, editors. (Ed.), *Human herpesvirus infections* (pp. 245–270). New York: Dekker.

- Glaser, R., Sheridan, J., Malarkey, W. B., MacCallum, R. C., & Kiecolt-Glaser, J. K. 2000. Chronic stress modulates the immune response to a pneumococcal pneumonia vaccine. *Psychosomatic Medicine*, 62, 804-807. doi:
- Goodman, R. 1997. The strengths and difficulties questionnaire: A research note. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, 38, 581-586. doi: 10.1111/j.1469-7610.1997.tb01545.x.
- Goodman, R., & Scott, S. 1999. Comparing the strengths and difficulties questionnaire and the child behavior checklist: Is small beautiful? *Journal of Abnormal Child Psychology*, 27, 17-24. doi: 10.1023/a:1022658222914.
- Graungaard, A. H., & Skov, L. 2007. Why do we need a diagnosis? A qualitative study of parents' experiences, coping and needs, when the newborn child is severely disabled. *Child Care Health and Development*, 33, 296-307. doi: 10.1111/j.1365-2214.2006.00666.x.
- Haarala, A., Kahonen, M., Lehtimäki, T., Aittoniemi, J., Jylhava, J., Hutri-Kahonen, N., et al. 2012. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: the Cardiovascular Risk in Young Finns Study. *Clinical and Experimental Immunology*, 167, 309-316. doi: 10.1111/j.1365-2249.2011.04513.x.
- Hahn, G., Jores, R., & Mocarski, E. S. 1998. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 3937-3942. doi: 10.1073/pnas.95.7.3937.
- Hastings, R. P., Daley, D., Burns, C., & Beck, A. 2006. Maternal distress and expressed emotion: Cross-sectional and longitudinal relationships with behavior problems of children with intellectual disabilities. *American Journal on Mental Retardation*, 111, 48-61. doi: 10.1352/0895-8017(2006)111[48:mdaeeec]2.0.co;2.
- Heaney, J. L. J., Phillips, A. C., & Carroll, D. 2012. Aging, health behaviors, and the diurnal rhythm and awakening response of salivary cortisol. *Experimental Aging Research*, 38, 295-314. doi: 10.1080/0361073x.2012.672134.
- Herrmann, C. 1997. International experiences with the hospital anxiety and depression scale - A review of validation data and clinical results. *Journal of Psychosomatic Research*, 42, 17-41. doi: 10.1016/s0022-3999(96)00216-4.
- Holt, P. G., & Keast, D. 1977. Environmentally induced changes in immunological function - acute and chronic effects of inhalation of tobacco smoke and other atmospheric contaminants in man and experimental animals. *Bacteriological Reviews*, 41, 205-216. doi:
- Hummel, M., & Abecassis, M. M. 2002. A model for reactivation of CMV from latency. *J Clin Virol*, 25 Suppl 2, S123-136. doi: S1386653202000884 [pii].
- Kiecolt-Glaser, J. K., Glaser, R., Shuttleworth, E. C., Dyer, C. S., Ogrocki, P., & Speicher, C. E. 1987. Chronic stress and immunity in family caregivers of Alzheimers-disease victims. *Psychosomatic Medicine*, 49, 523-535. doi:
- Kiecolt-Glaser, J. K., Speicher, C. E., Glaser, R., Dura, J. R., & Trask, O. J. 1991. SPOUSAL CAREGIVERS OF DEMENTIA VICTIMS - LONGITUDINAL CHANGES IN IMMUNITY AND HEALTH. *Psychosomatic Medicine*, 53, 345-362. doi:
- Kleinman, D., Sarov, I., & Insler, V. 1986. REACTIVATION OF CYTOMEGALOVIRUS IN ENDOMETRIAL CELLS BY ESTRADIOL. *Gynecologic and Obstetric Investigation*, 21, 136-143. doi:
- Licastro F., C., G., Lio D., Porcellini, E., Colonna-Romano G., Franceschi C., Caruso, C. 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. *Immun Ageing*, 2, 8. doi:

- Marmot, M. G., Davey-Smith, G., Stansfield, S., Patel, C., North, F., Head, J., et al. 1991. Health inequalities among British civil servants: the Whitehall II study. *Lancet*, 337, 1387-1393. doi:
- McVoy, M. A., & Adler, S. P. 1989. Immunologic evidence for frequent age-related Cytomegalovirus reactivation in seropositive immunocompetent individuals. *The Journal of Infectious Diseases*, 160, 1-10. doi:
- Moorey, S., Greer, S., Watson, M., Gorman, C., Rowden, L., Tunmore, R., et al. 1991. The factor structure and factor stability of the hospital anxiety and depression scale in patients with cancer. *British Journal of Psychiatry*, 158, 255-259. doi: 10.1192/bjp.158.2.255.
- Olsson, J., Wikby, A., Johansson, B., Lofgren, S., Nilsson, B. O., & Ferguson, F. G. 2000. Age-related change in peripheral blood T-lymphocyte subpopulations and cytomegalovirus infection in the very old: the Swedish longitudinal OCTO immune study. *Mechanisms of Ageing and Development*, 121, 187-201. doi:
- Pariante, C. M., Carpiniello, B., Orru, M. G., Sitzia, R., Piras, A., Farci, A. M. G., et al. 1997. Chronic caregiving stress alters peripheral blood immune parameters: The role of age and severity of stress. *Psychotherapy and Psychosomatics*, 66, 199-207. doi:
- Pass, R. F., & Hutto, C. 1986. GROUP DAY-CARE AND CYTOMEGALOVIRAL INFECTIONS OF MOTHERS AND CHILDREN. *Reviews of Infectious Diseases*, 8, 599-605. doi:
- Pawelec, G., Akbar, A., Caruso, C., Solana, R., Grubeck-Loebenstein, B., & Wikby, A. 2005. Human immunosenescence: is it infectious? *Immunological Reviews*, 205, 257-268. doi: 10.1111/j.0105-2896.2005.00271.x.
- Pawelec, G., & Derhovanessian, E. 2011. Role of CMV in immune senescence. *Virus Research*, 157, 175-179. doi: 10.1016/j.virusres.2010.09.010.
- Pawelec, G., Derhovanessian, E., Larbi, A., Strindhall, J., & Wikby, A. 2009. Cytomegalovirus and human immunosenescence. *Reviews in Medical Virology*, 19, 47-56. doi: 10.1002/rmv.598.
- Phillips, A. C., Burns, V. E., Carroll, D., Ring, C., & Drayson, M. 2005. The association between life events, social support and antibody status following thymus-dependent and thymus-independent vaccinations in healthy young adults. *Brain Behav Immun*, 19, 325-333. doi:
- Provinciali, M., Moresi, R., Muzzioli, M., Tarabelli, D., Sirolla, C., Melchiorre, M. G., et al. 2004. Psychological, neuroendocrine and immune measures in non spousal carers of disabled elderly in Italy. *Neuroendocrinology Letters*, 25, 391-396. doi:
- Rasmussen, L. E. 1991. Gene products of cytomegalovirus and their immunologic significance. In H. M (Ed.), *Cytomegalovirus: biology and infection* (2nd ed.): New York: Plenum Medical Book Company.
- Riddell, S. R., & Greenberg, P. D. 1997. T cell therapy of human CMV and EBV infection in immunocompromised hosts. *Reviews in Medical Virology*, 7, 181-192. doi: 10.1002/(sici)1099-1654(199709)7:3<181::aid-rmv200>3.0.co;2-w.
- Savva G M, P. A., Kaul B, Morgan K, Huppert F A, Brayne C, Moss P H A. 2013. Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell*, 1-7. doi: Doi: 10.1111/accel.12059.
- Simanek, A. M., Dowd, J. B., Pawelec, G., Melzer, D., Dutta, A., & Aiello, A. E. 2011. Seropositivity to Cytomegalovirus, Inflammation, All-Cause and Cardiovascular Disease-Related Mortality in the United States. *Plos One*, 6 doi: 10.1371/journal.pone.0016103.
- Sopori, M. 2002. Effects of cigarette smoke on the immune system. *Nature Reviews Immunology*, 2, 372-377. doi: 10.1038/nri803.

- Spira, A. P., Beaudreau, S. A., Stone, K. L., Kezirian, E. J., Lui, L. Y., Redline, S., et al. 2012. Reliability and Validity of the Pittsburgh Sleep Quality Index and the Epworth Sleepiness Scale in Older Men. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences*, 67, 433-439. doi: 10.1093/gerona/qlr172.
- Strandberg, T. E., Pitkala, K. H., & Tilvis, R. S. 2009. Cytomegalovirus Antibody Level and Mortality Among Community-Dwelling Older Adults With Stable Cardiovascular Disease. *Jama-Journal of the American Medical Association*, 301, 380-382. doi:
- Trzonkowski, P., Mysliwska, J., Pawelec, G., & Mysliwski, A. 2009. From bench to bedside and back: the SENIEUR Protocol and the efficacy of influenza vaccination in the elderly. *Biogerontology*, 10, 83-94. doi: 10.1007/s10522-008-9155-5.
- Vasto, S., Colonna-Romano, G., Larbi, A., Wikby, A., Caruso, C., & Pawelec, G. 2007. Role of persistent CMV infection in configuring T cell immunity in the elderly. *Immunity & ageing : I & A*, 4, 2. doi: 10.1186/1742-4933-4-2.
- Vedhara, K., Cox, N. K. M., Wilcock, G. K., Perks, P., Hunt, M., Anderson, S., et al. 1999. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet*, 353, 627-631. doi: 10.1016/s0140-6736(98)06098-x.
- Vedhara, K., McDermott, M. P., Evans, T. G., Treanor, J. J., Plummer, S., Tallon, D., et al. 2002. Chronic stress in nonelderly caregivers - Psychological, endocrine and immune implications. *Journal of Psychosomatic Research*, 53, 1153-1161. doi: Pii s0022-3999(02)00343-4
10.1016/s0022-3999(02)00343-4.
- Wall, N. A., Chue, C. D., Edwards, N. C., Pankhurst, T., Harper, L., Steeds, R. P., et al. 2013. Cytomegalovirus Seropositivity Is Associated with Increased Arterial Stiffness in Patients with Chronic Kidney Disease. *Plos One*, 8 doi: 10.1371/journal.pone.0055686.
- White N, H., RP. 2004. Social and professional support for parents of adolescents with severe intellectual disabilities. *Journal of Applied Research in Intellectual Disabilities*, 17, 181-190. doi:
- Yamashita, Y., Fujimoto, C., Nakajima, E., Isagai, T., & Matsuishi, T. 2003. Possible association between congenital cytomegalovirus infection and autistic disorder. *Journal of Autism and Developmental Disorders*, 33, 455-459. doi: 10.1023/a:1025023131029.
- Zarit, S. H., Reever, K. E., & Bachpeterson, J. 1980. Relatives of the impaired elderly - correlates of feelings of burden. *Gerontologist*, 20, 649-655. doi:
- Zigmond, A. S., & Snaith, R. P. 1983. The hospital anxiety and depression scale. *Acta Psychiatrica Scandinavica*, 67, 361-370. doi: 10.1111/j.1600-0447.1983.tb09716.x.